

poles at the terminal portions of the U straddle the sorting region (e.g., above and below or in the same plane).

[0061] In certain embodiments, the permanent magnet provides a field strength of between about 0.01 and 1 T, such as between about 0.1 and 0.5 T. Note that for some exotic applications, it may be appropriate to use stronger magnetic fields such as those produced using superconducting magnets, which may produce magnetic fields in the neighborhood of about 5 T.

[0062] Permanent magnets are made from ferromagnetic materials such as nickel, cobalt, iron, alloys of these and alloys of non-ferromagnetic materials that become ferromagnetic when combined as alloys, known as Heusler alloys (e.g., certain alloys of copper, tin, and manganese). Many suitable alloys for permanent magnets are well known and many are commercially available for construction of magnets for use with the present invention. A typical such material is a transition metal-metalloid alloy, made from about 80% transition metal (usually Fe, Co, or Ni) and a metalloid component (boron, carbon, silicon, phosphorus, or aluminum) that lowers the melting point. Permanent magnets may be crystalline or amorphous. One example of an amorphous alloy is $\text{Fe}_{80}\text{B}_{20}$ (Metglas 2605).

[0063] In a specific embodiment disclosed herein (an embodiment of the FIG. 1 design), the external magnetic field is provided by a pair of 5 millimeter diameter NdFeB magnets (K&J Magnetics, Jamison, Pa.) attached to the top and bottom sides of a sorting region in a microfluidic device.

[0064] In one specific embodiment that has been designed and built, the field gradient produced by the MFG was approximately 5000 T/m within 1 micrometer from the edge of the MFG. This device employed two 5 mm diameter external NdFeB magnets (K&J Magnetics, Jamison, Pa.) attached to the top and bottom sides of a sorting region. The MFG was provided as series of parallel strips, each having a thickness of about 0.2 micrometer, a width of about 20 micrometers. The individual strips were separated from one another by about 20 micrometers. Further, the angle of MFG strips was about 28° with respect to the flow direction.

[0065] The magnetic capture particles employed in microfluidic separations of this invention may take many different forms. In certain embodiments, they are superparamagnetic nanoparticles, although in some cases they may be ferromagnetic or paramagnetic.

[0066] As a general proposition, the magnetic particles should be chosen to have a size, mass, and susceptibility that allows them to be easily diverted from the direction of fluid flow when exposed to a magnetic field in microfluidic device (balancing hydrodynamic and magnetic effects). In certain embodiments, the particles do not retain magnetism when the field is removed. In a typical example, the magnetic particles comprise iron oxide (Fe_2O_3 and/or Fe_3O_4) with diameters ranging from about 10 nanometers to about 100 micrometers. However, embodiments are contemplated in which even larger magnetic particles are used. For example, it may be possible to use magnetic particles that are large enough to serve as a support medium for culturing cells.

[0067] Note that aggregation of magnetically labeled cells due to mutual magnetic interactions is generally undesirable, and may be avoided by using superparamagnetic nanoparticles. Thus, the magnetic particles may be commonly formed from single-domains of a ferromagnetic material (<100 nm iron oxide particles). In the absence of an external field, due to their small size, the thermal energy is sufficient to randomly

orient their magnetization, resulting in a negligible average magnetization even below the Curie temperature. When an external field is applied, the magnetization of these superparamagnetic beads saturates at a relatively weak external magnetic field of ~ 0.02 T.

[0068] The magnetic particles may be coated with a material rendering them compatible with the microfluidics environment and allowing coupling to particular target components. Examples of coatings include polymer shells, glasses, ceramics, gels, etc. In certain embodiments, the coatings are themselves coated with a material that facilitates coupling or physical association with targets. For example, a polymer coating on a micromagnetic particle may be coated with an antibody, nucleic acid sequence, avidin, or biotin.

[0069] One class of magnetic particles is the nanoparticles such as those available from Miltenyi Biotec Corporation of Bergisch Gladbach, Germany. These are relatively small particles made from coated single-domain iron oxide particles, typically in the range of about 10 to 100 nanometers diameter. They are coupled to specific antibodies, nucleic acids, proteins, etc.

[0070] Another class of magnetic particles is made from magnetic nanoparticles embedded in a polymer matrix such as polystyrene. These are typically smooth and generally spherical having diameters of about 1 to 5 micrometers. Suitable beads are available from Invitrogen Corporation, Carlsbad, Calif. These beads are also coupled to specific antibodies, nucleic acids, proteins, etc.

[0071] It should be understood that certain embodiments make use of intrinsic magnetic properties of the sample material. In such embodiments, magnetic particles need not be employed. Examples of such materials include erythrocytes, small magnetic particles for industrial applications, etc.

[0072] Flow Systems and Hydrodynamics

[0073] Generally, the physical separation between the labeled and unlabeled sample components occurs through the balance between hydrodynamic and magnetophoretic forces. As the magnetically labeled components travel within the microfluidic channel, there exists a hydrodynamic drag force (F_d). Assuming that the magnetically labeled component is spherical, then the force can be approximated as $F_d = 6\pi\eta R_p v$, where η is the viscosity of the medium, R_p is the particle radius and v is the velocity. When the labeled component passes over the MFGs at an angle as shown in FIG. 1, the MFG imposes an attractive magnetophoretic force (F_m). In this case, if the component of F_d perpendicular to the MFG is less than the value of F_m (i.e., $F_d \sin \theta < F_m$), then the velocity vector of the labeled cell will be significantly modified in the direction parallel to the MFG pattern, as demonstrated in FIG. 1.

[0074] In view of this, the linear fluid velocity in the sorting regions should be controlled to provide proper balance with the magnetic gradient size to ensure that efficient sorting can be accomplished. For a CMACS sorting region as depicted in FIG. 1, for example, a fluid velocity of approximately 3 mm/s has been found to be appropriate. At this velocity, viscous drag forces on Dynal M280™ 2.8 μm microbeads are expected to be ~ 160 pN (with $\eta \sim 0.002 \text{ kg}\cdot\text{m}^{-1}\cdot\text{s}^{-1}$ for the suspension medium). For a MFG oriented at approximately 28° with respect to the fluid direction and producing a field gradient of approximately 5000 T/m in the sorting region, a 3 mm/s allows efficient sorting. General ranges of fluid velocity suitable for use with various embodiments of the invention will be presented below.